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This project has developed a system of biological detection at standoff using Raman lidar, a well-known method of monitoring atmospheric gases and pollutants [1]. One application of a biological agent standoff detector would be in the advanced warning of a Bacillus anthracis attack. In this work we explore the feasibility of a Raman lidar system for the remote sensing of biological agents, specifically bacterial endospores.

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Final Report

TOWARD THE REMOTE SENSING OF BACTERIAL ENDOSPORES BY RAMAN LIDAR

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October 26, 2004

TOWARD THE REMOTE SENSING OF BACTERIAL ENDOSPORES BY RAMAN LIDAR

Final Report for research supported by the U.S. Army Research Office

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Abstract

This project has developed a system of biological detection at standoff using Raman lidar, a well-known method of monitoring atmospheric gases and pollutants [1]. One application of a biological agent standoff detector would be in the advanced warning of a *Bacillus anthracis* attack. In this work we explore the feasibility of a Raman lidar system for the remote sensing of biological agents, specifically bacterial endospores.

1. Introduction

For this project we have used Bacterial endospores of the genus *Bacillus* containing a high mass fraction of the calcium salt of 2, 6-pyridinedicarboxylic acid (CaDPA). CaDPA is concentrated within the endospore core, and comprises from 7% to13.5% of the total spore mass, depending on *Bacillus* species [2]. CaDPA is not present in background atmosphere (i.e., dust or pollens) and is therefore an excellent candidate analyte for the detection of *Bacillus* spores, within a Raman lidar system. The presence of CaDPA would then be indicative of a *Bacillus anthracis* biological weapon attack, but would have to be followed by a secondary more specific technique for positive species identification.

CaDPA has been investigated in prior Raman studies, and has been found to have Raman-active modes from 28 cm^{-1} through 3084 cm^{-1}[3]. Not all features are unique to CaDPA, however. For example, the 3084 cm^{-1} corresponds to an aromatic carbon-hydrogen stretching vibration observed for many organic compounds. CaDPA-specific Raman features are more likely restricted to the region below 800 cm^{-1}, particularly the THz offset region from 0 to 300 cm^{-1}. Raman spectra of intact endospores above 450 cm^{-1} show features clearly consistent with the presence of CaDPA, [4], however there is a lack of experimental data in and around the THz region.

2. Phenomenology

We have used *ab initio* quantum mechanical calculations (Gaussian program package, density functional theory with the B3LYP functional, frequencies scaled by 0.89) to predict the normal mode frequencies, IR and Raman activities and IR intensities of CaDAP monomers and dimers. Shown in Fig. 1 are the calculated modes of a DPA dimer. The 44, 72 and 183 cm^{-1} modes are predicted to be Raman active, and would

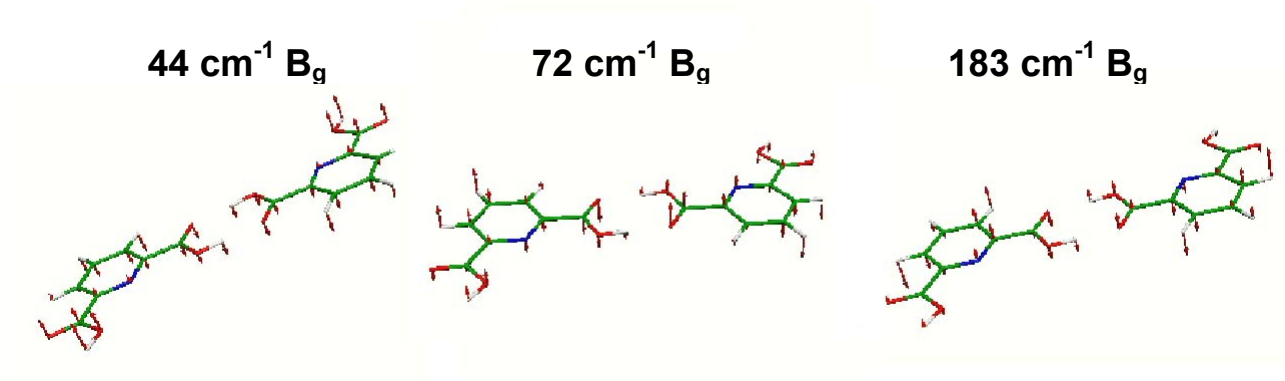

Figure 1: THz region vibrational modes of 2, 6-pyridinedicarboxylic acid (DPA) dimer.

3. Raman Detection of DPA: System One

A Raman spectrometer as shown in Figure 2 is being used to make bistatic measurements of 2, 6-pyridinedicarboxilic acid (DPA) crystals. The spectrometer consists of a Q-switched Nd:YAG laser pump emitting 1.064 μm with a pulse width of 8 ns, a repetition frequency of 10 Hz, pulse energy of 250 mJ, and a peak power of about 10 MW. The output beam from the Nd:YAG laser is a TEM_{00} Gaussian mode having a large enough output spot diameter ($2\omega_0 \sim 6$ mm) and long enough Rayleigh length ($z_0 \sim$ 100 m) that the beam is practically collimated for the present (bench-top) experiments. The beam is directed over a path length of approximately 1 m to the DPA sample without focusing where it scatters omni directionally. A free-space-to-fiber coupler is placed approximately 10 cm from the sample and at an angle of 90 degrees with respect to the pump beam. The coupler consists of a microscope-objective lens train and an overmoded fiber with entrance aperture placed in the focal plane. The overmoded fiber facilitates the

alignment procedure and allows for high coupling efficiency into the optical spectrum analyzer (OSA). The OSA is a Model 6317C from Ando having a maximum resolution of 0.01 nm and a noise floor of -70 dBm at this resolution.

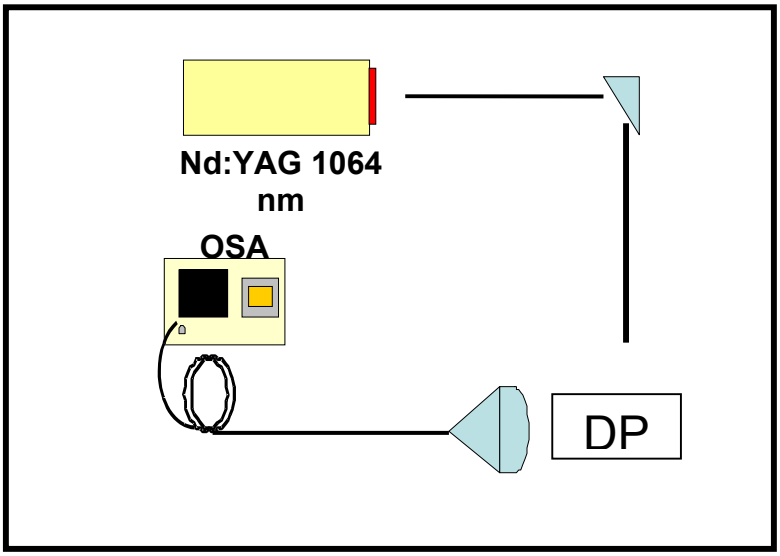

Figure 2: Raman Lidar with OSA Receiver used for Resolving Scatter DPA Spectra

4. Raman Measurement Results

The initial Raman spectral results for this set-up are plotted in Fig. 3. They agree qualitatively with the results of Carmona [3] for the Raman shift at 230 cm^{-1}. By fitting a Lorentzian distribution to this Raman peak, it is estimated that there is approximately 2 nW of power in this band. Using this power we can estimate the cross section of DPA at 230 cm^{-1}. This critical number gives us our first estimate of the intensity of scattered Raman light: something not discussed in prior work and a limitation on the range of a remote sensing Raman lidar. The phenomenology is described by the target reflectivity equation, valid in the limit of low concentration N [2]:

$$\frac{\rho}{\pi} \sim \frac{c\tau}{2} \cdot \frac{N\sigma_{ram}}{4\pi}$$

where :

$\frac{\rho}{\pi}$ is target reflectivity

c is the speed of light

τ is the duration of the laser pulse

σ_{ram} is the Raman cross section

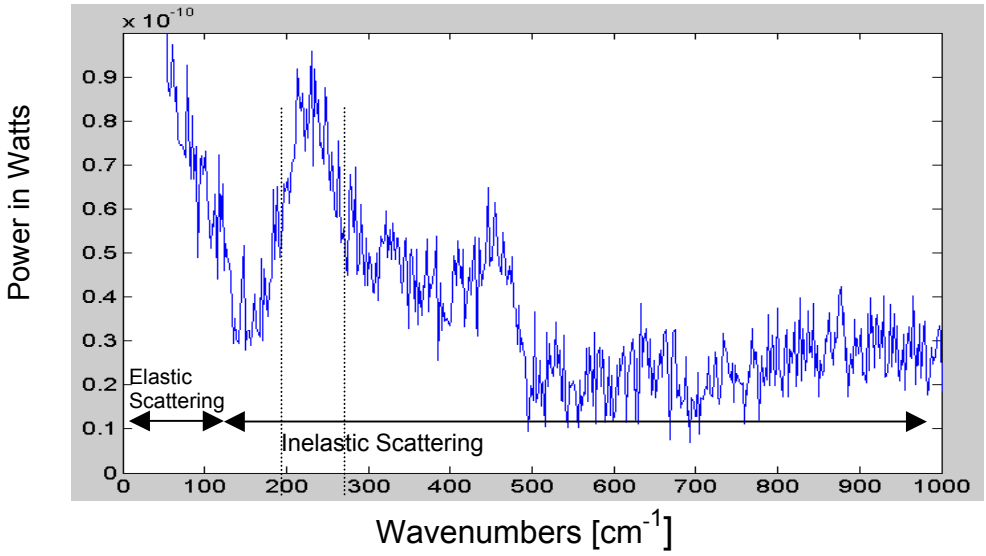

Figure 3: Preliminary Results from Raman Scattering Experiments

When combined with the single scattered lidar equation [2], this gives the reflectivity versus range estimation shown in Fig. 4. This curve estimates the minimum detectable concentration levels at a given range, assuming a receiver signal-to-noise of unity. Such predictions are not specifically made here but are left in terms of reflectivity due to the preliminary nature of the cross section measurements. Additional experiments are being carried out for complete confirmation of these measurements, and of prior work. This has been hindered by two effects: (1) The low sensitivity of the OSA as compared to photon shot-noise limited detectors [e.g.,. liquid nitrogen cooled Ge avalanche photodiode (APD)], and (2) the low opto-electrical conversion efficiency of the OSA due to its limiting post-detection bandwidth of 45 KHz compared to 50 MHz for the signal (roughly corresponding to the –3 dB electrical bandwidth of the Gaussian pulse).

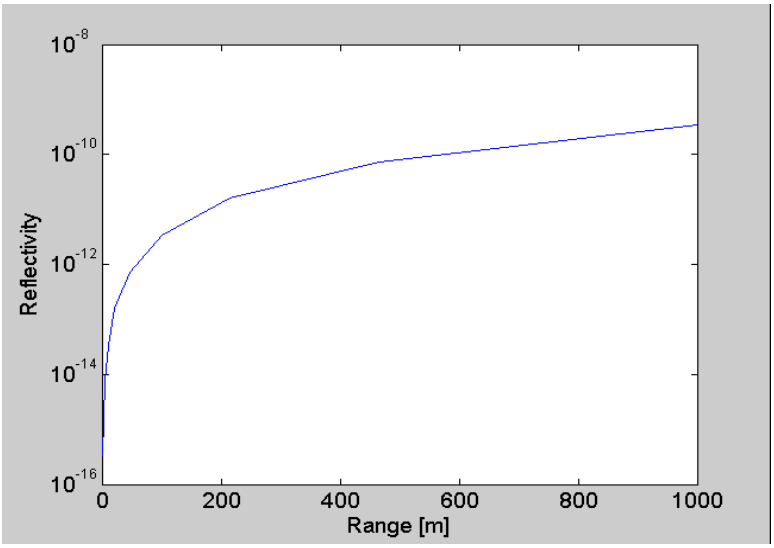

Figure 4: Theoretical Detection Limits using lidar equation

5. Raman Detection of DPA: System Two

To counter the deficiencies of the simple setup described above, we assembled an improved version shown in Fig. 5. This system addresses the smaller power in the bands by increasing the sensitivity with germanium det box car integration. It also aims to retain the selectivity of wavelength.

$$\left(\frac{S}{N}\right)_P = \frac{i_s^2}{\left\langle i_{shot}^2 \right\rangle + \left\langle i_{dark}^2 \right\rangle}$$

$$= \frac{R \cdot P_{signal}^2}{2ei_{signal}B + 2ei_{dark}B}$$

-Grating efficiency

$$NEP_{shot} = \sqrt{2ei_{signal}}$$

$$NEP_{dark} = \sqrt{2ei_{dark}}$$

The second system will be implemented in the same way as the previous system; however, the OSA is replaced by a dual-grating spectrometer, a Ge APD, and a boxcar integrator. A 1-meter-path dual-grating spectrometer has been retrofitted with gratings blazed for the Nd:YAG wavelength (1064 nm), increasing the throughput efficiency in the wavelengths of interest by >3 dB, while maintaining sub-nm wavelength resolution. This throughput and resolution should allow the measurement of fine THz offset (sub 300 cm^{-1} or less than 9 THz) Raman features without interference from elastic (Rayleigh) light scattering.

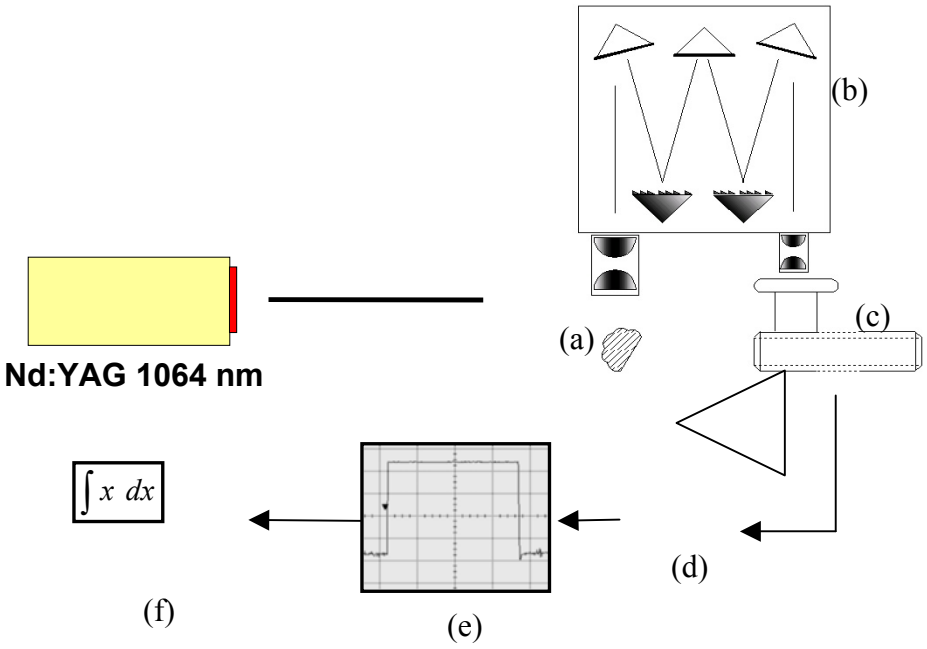

Figure 5: 2nd proposed system. Shown are the crystalline DPA sample (a), the dual grating spectrometer (b), the liquid nitrogen dewar containing the Ge APD (c), the amplifying electronics (d), the gating function of the boxcar integrator (e), and the integrating function of the boxcar integrator (f).

To further improve our ability to measure the weak Raman light, a high-sensitivity, high-bandwidth (400 MHz), liquid nitrogen cooled, Ge APD (NEP ~$1x10^{-13}$ $W/Hz^{1/2}$) will be used to collect these inelastically scattered photons. This will both improve our sensitivity and increase our bandwidth over the OSA. The signals produced by the detector are amplified using transimpedance and voltage gain stages. The

amplifying electronics are being designed and simulated in ADS (Agilent's Advanced Design System), which predicts a transimpedance gain of 10^7 V/A and a noise figure of 4.0. Better designs are being explored to reduce this noise figure while maintaining the high gain.

The amplified signal will be gated and time-averaged using a boxcar integrator. This will allow us to collect photoelectrons only during the short pulse duration of the Q-switched Nd:YAG laser while ignoring background- and dark-photoelectron noise. Additionally, it will allow the averaging of signals, increasing the signal-to-noise ratio by $N^{1/2}$ where N is the number of pulses.

Conclusion

Through this research, we have made preliminary measurements of inelastic light scattering from DPA using an Nd:YAG pump laser and an optical spectrum analyzer, and have observed scattering peaks that we attribute to low-energy Raman scattering. This has encouraged us to engineer a new system that improves both sensitivity and wavelength resolution. This system will allow us to accurately measure the Raman cross section of DPA, and ultimately construct a sensor that can detect bioparticles at useful stand-off.

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